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STUDY AICOHOLIC EXTRACT EFFECTING OF ORANGE PLANT IN THE GROWTH OF TWO TYPES OF ALGAE MICROCYSTIS SP. & CHROOCOCCUS SP

ZAINAB MOHSIN IBRAHIM, MUROOJ ABBAS BUHLOOL,
IQBAL AZEEZ AMEEN & IHSAN HAMEED KHUDHAIR
Department of Biology, College of Science, Thi-Qar University, Iraq

ABSTRACT

The main objective of this study is examine the effect of Bitter Orange (*Citrus aurantium* L.) Peel extract against growth two species of blue-green algae (Cyanobacteria): *Chroococcus* sp and *Microcystis* sp. the growth rate of algae counted directly by Chamber. The algae farms were divided in to five groups as following : the first group(C1): Treatment control concentration 0% , the second group(C2): Treatment of alcoholic extract concentration of the orange plant 1% , the third group(C3): alcoholic extract concentration of the orange plant 3% , the fourth group (C4): Treatment of alcoholic extract concentration of the orange plant 5% , the fifth group(C5): Treatment of alcoholic extract concentration of the orange plant 8% . All groups were noted during the time periods (today's second, the fourth day, the eighth day and the tenth day).

The results showed the Bitter Orange (*Citrus aurantium* L.) Peel extract caused a significant inhibition($p<0.05$) in the rate of growth of algae at concentration 8% for two species, and the colonies were reached in *Microcystis* sp (81) cell in ml compared with control , in *Chroococcus sp.* was least cells (43). *Microcystis* sp was more resistance for Bitter Orange Peel extract.

KEYWORDS: Cyanobacteria, Chroococcus Algae, Microcystis Algae, Bitter Orange (Citrus Aurantium L.) Peel Alcoholic Extract

INTRODUCTION

Considered algae fundamental basic major biological cycles and capacity spread received considerable attention since the invention of the Microscope and so far, and contribute to algae process of self-purification in bodies of water through Phytosynthesis where launches dissolved oxygen gas leading to the perpetuation of gaseous balance between oxygen and dioxide carbon between the air and water (Saadi and Suleiman, 2006). and it has important biological, medical and economic which is necessary to sustain life (Mouloud *et al*., 1990), Although the benefits of algae, but plentiful in the aquatic environment causing pollution (Eutrophication phenomenon) as well as some of them toxic (Saadi and Suleiman 2006).

The pollution of the problems facing the world, especially water pollution, which is defined as the change in the river's water conditions directly or indirectly as a result of the activities of the human problem and thus it causes changes in environmental ecosystems and constitutes a danger to human health, can some types of algae live in water contaminated with feces sewage, as used large amounts of nitrogen compounds and phosphates in waste during its growth (sprue, 1992). Nutrient direct impact on the density of phytoplankton, is the large increase in nutrients.

The large increase in plant nutrients (nitrogen and phosphorus) in the water of the problems that threaten the water ecosystem is, and this increase grow large numbers of algae and prosperity on the surface of the water layer, causing Algale Blooming phenomenon and which are effects adverse to the aquatic environment and organisms that live in them and so lead to a reduction in the diversity of algae (Klug ,2003), and that the viability of algae make the water taste and smell is not good, and it has the ability to change the pH and water color and turbidity. Considering algae from organisms that change the chemical and physical properties important to the water, such as turbidity, temperature, color and materials radioactive and organic materials, bio-oxygen demand (BOD) and pH and dissolved oxygen (DO) (Perscott, 1975). The use of some immune extracts found in some fruits and vegetables that feed the human in the daily food oranges protect the body from damage resulting from toxins microbiology because they contain effective groups at high rates have the ability to protect body cells from damage these toxins by increasing the production of enzymes antioxidants when strep (Freeman, and Kodera, 1995; Lawson and Hughes, 1992) These are rich source of vitamin-C (Ascorbic acid). The peel of the citrus fruits contains various active constituents and essential oils. Orange peel is one of the important dietary sources of antioxidant phenolics (Jayaprakasha et al., 2008)

Citrus is one of the most important commercial fruit crops grown in all continents of the world. Oranges is an importantmedicinalplant of the family Rutaceae. It is cultivated mainly for its alkaloids, which are having anticancer activities and the antibacterial potential in crude extracts of different parts (awaii *et al.*, 2000). The peel of Citrus fruits is a rich source of flavonoid glycosides, coumarins, βand γ- sitosterol, glycosides and volatile oils (Shahnah *et al*., 2007). Many polymethoxylated flavones have several important bioactivities, which are very rare in other plants (Ahmad *et al*., 2006). In addition the fiber of citrus fruit also contains bioactive compounds, such as polyphenols, the most important being vitamin C (or ascorbic acid), and they certainly prevent and cure vitamin C deficiency-the cause of scurvy (Aronson, 2001).

The aim of the study to know the vital effects of alcoholic extract of orange peel, in terms of the fact that this extract Stimulator or an inhibitor of the growth of two types of algae Cyanophayta namely: *Chroococcus* sp. & *Microcystis* sp.

MATERIAL AND METHODS

Washing and Sterilizing Instruments used:

Washed and sterilized all-glass tools used in the media culture preparation and in the isolation, purification and cultivation of microalgae acid, hydrochloric acid HCl concentration (20%) then washed tools tap water and then distilled water was dried and sterilized using electric oven at a temperature of (105)°C for two hours were used flasks conical glass various sizes (250, 500) ml in culture experiments after closed at clean and sterile cotton, of the agar media have been used sterile plastic dishes

Sampling

Subsurface water samples were collected from the Different areas of the River Gharraf, the samples were collected by using clean polyethylene bottles, it installed to sample part using formalin concentration of 4% for the purpose of microscopic examination while leaving the other without installed for the purpose of of culture.

Media Culture

Attended the media (Chu -10) and the modefied from (Al- Aarajy, 1996) in Stock solutions Table (1) and use distilled water in the preparation and without sterilization while using it and save the media in the refrigerator at a temperature(4°c)in dark.

The mixed equal amounts (1) mL of each Stock solutions and then complete the volume to 1 liter with distilled water then justice the pH between (7.4 - 7) by adding drops of sodium hydroxide solution NaOH concentration of 10 mg / L or acid Hydrochloric HCl concentration (10%) using pH - meter and infertility media in Autocleave under temperature (121) ° C and pressure (15) lbs / $inch^2$ for 20 minutes, then leave to cool degree lab temperature then phosphate salts added to it after sterilized by filtration using filtration paper their openings diameter (0.45) microns to prevent the deposition of phosphate on the walls of the glass bottle during sterilization, The culture media solid has attended the same liquid media components after the addition of Agar him by 15 g / liter, and sterility and left to cool then pour in the petri dishes dry and sterile near flame burner and preserved all the dishes after hardening in the refrigerator temperature (4) ºC in the upside-down while in use.

Table 1: The Chemical Composition of the Media (Chu -10) Modified by (Al – Aarajy, 1996)

mg /l	Compound	mg/l	Compound
25	NaHCO3	53.3	NaNO3
0.045	MnCl2. 4H2O	10	K2HPO4
0.007	NH4)6Mo7O24. 4H2O(	25	MgSO4. 7H2O
0.056	ZnSO4.7H2O	40	CaCl2. 2H2O
0.02	CuSO4. 6H2O	1.46	FeCl3. 6H2O
0.72	H3BO3	6.2	Na2SiO3. 9H2O
0.01	CoCl2. 6H2O	31.8	Na2EDTA

Isolation and Purification of Algae:

For order to obtain unialgal Culture Used dishes agar planning method and after a series of dilutions for a unialgal Culture (Stein, 1973).The purification of unialgal Culture from bacteria, according to (Wilson and Demmig-Adams, 2007; Kyung and Lee, 2001) described detail in (Pereira1 *et al*.,2006).

Diagnosis Species of Algae

The following sources adopted in the diagnosis of species of algae used in the study: (Gowda et al, 2004; Kivanc and Kunduhoglu, 1997; Topal, 1989) Where.it.Was.isolated.algae Classified and described below Division

Cyanophyta (Blue green algae)

Class: Cyanophyceae

Order: Chroococcales

Family:Chroococcaceae

Genus:Chroococcus sp., Microcystis sp

EXTRACTION PROCEDURE

The plants used in this study were Citrus Aurantium L.(Bitter Orange) The peels were collected from the local fruit juice shops. After collection, the peels were shade dried at room temperature (30 - 35ºC). 20 gm of peels of oranges were coarsely powdered using a mortar and pestle and were further reduced to powder using an electric blender. The powder was transferred into closed containers for further use.

The dried and powdered peel materials (10 gm) were extracted with 200 ml of each solvent separately by using soxhlet extractor for 2 to 5 h at a temperature not exceeding the boiling point of the Solvent. The solvents used for the study were ethanol. The extracts were filtered and then concentrated to dryness. The extract were transferred to glass vials and kept at 4º C before use. The extracts were dissolved in 25% aqueous dimethyl sulfoxide (DMSO) to produce a stock solution of 100 mg/ml. Ladd *et al.* (1978)

It was prepared concentrations of alcoholic extract of the plant orange was calculated by the following formula:

N1 * V1 = N2 * V2

Whereas

Treatment control concentration 0% C1:

C2: Treatment of alcoholic extract concentration of the orange plant 1%

C3: Treatment of alcoholic extracts concentration of the orange plant 3%

C4: Treatment of alcoholic extracts concentration of the orange plant 5%

C5: Treatment of alcoholic extracts concentration of the orange plant 8%

Testing of Extracts Activity for (Peel Bitter Orange) for Growth Rate of Algae

Appoint isolates the addition of (0.10) from the farm pure liquid inoculums for each of the two algae to volumetric flasks (250 ml) container at media culture supported by alcoholic extract of pomegranate previous concentrations above, and using three replicates per concentration, incubter at a temperature (27 ± 2)° C with the level of lighting (50) µE / m 2 / s and system Lighting (16: 8) Lighting: darkness bearing in mind the continuous shaking of samples a day for the purpose of obtaining the desired growth (Tomaselli *et al*., 1981), as well as the sample Culturing without adding alcoholic extract Orange of her mind in order to control sample

Measuring the Rate of Growth

The growth rate of algae counted directly by Chamber Shidu(Coombs et al.,1986)

RESULTS

The results of the current study showed a decrease in the number of cells *Chroococcus* sp. and *Microcystis* sp. With increasing concentrations of alcoholic extract (peel)Bitter Orange and gradually during the period of incubation as in Figure (1.2) , and confirmed by the statistical analysis of the existence of a negative relationship between the number of cells and increasing concentrations of alcoholic extract (peel) Bitter Orange.

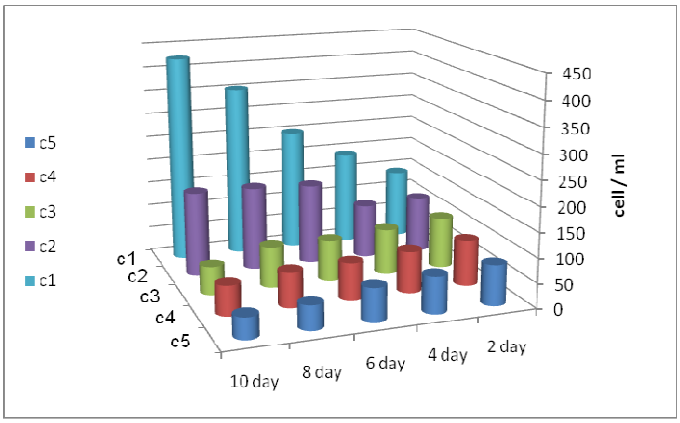

Figure 1: Role of Alcoholic Extract (Peel) Citrus Aurantium L (Bitter Orange) in Growth Rate of Chroococcus sp

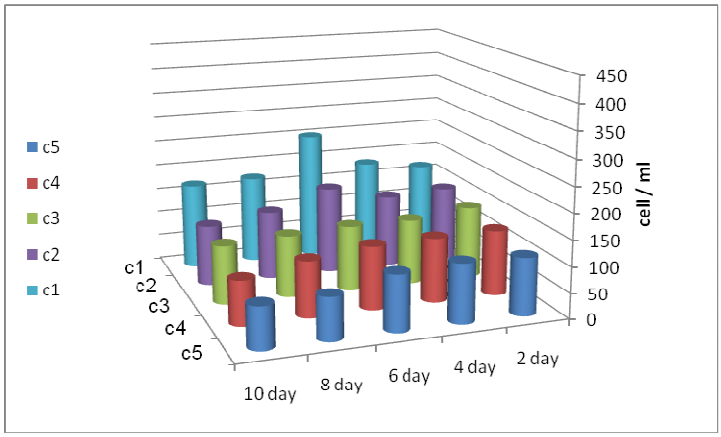

Figure 2: Role of alcoholic extract (peel) Citrus aurantium L (Bitter Orange) in growth rate of Microcystis sp

The results of the present study shown in figure (1, 2). The result showed the first day a significant Resistant ($p<0.05$) in growth of rate of *Microcystis* sp of alcoholic extract (peel) Bitter Orange more than *Chroococcus* sp , Where the rate of growth at calculation found that the highest number of algae cells *Chroococcus* sp. When treatment to differing concentrations of alcoholic extract (orange peel) (425cells / ml when the treatment C1 (0%) in the tenth day of treatment and the lowest number was (43) cells / ml when the treatment C5 (8%) on the same day , either *Microcystis* sp. has reached a higher number of cells (174) cells / ml when the treatment C1 (0%) in the eightth day also and the lowest number (81) cells / ml when the treatment C5 (8%) on the tenth day showed the results of the statistical analysis and the presence of significant differences ($P\leq0.05$) between treatments and between periods of incubation.

DISCUSSIONS

Widespread use of the two genus in the our environment and local bluegreenalgae from being encouraged to isolate and purify and study the effect of alcoholic extract (orange.Peel Results showed isolation from the local environment and the spread of the species and genus of algae, mostly of blue-green algae and diatoms, and between these genuses is more Chroococcus sp. A unicellular alga assembles in colonies and characterized cells form spherical and bethe contents of the cell to an area distinct dark and light area.

The blue-green alga Microcystis sp. It is also present in our environment, but it will be longer growth rate than growth rate Chroococcus sp.And present in the colonies also because the colony be either regular or elongated or circular or irregular, a toxic algae because it produces toxic compound Microcystin a polypeptide as mg per kg body causing death (al-Saadi & Sulaiman, 2006)

Indicate the negative relationship between the concentrations of alcoholic extract of (orange peel) and the rate of growth two algae to influence the inhibitory extract may be due to the effectiveness of oranges against two algae to fit on a number of effective compounds against microorganism such as compounds alkaloid, flavonoid, glycosides , polyphenol , tannin(Al- Brahim 2008; Lu *et al*. 2002; Ahmad & Beg. 2001)

The resistance may be due to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism (Abu-Shanab et al.,2004).

Another reason may be due to the fact that these two algae belongs to a class of blue- green algae which resemble bacteria in many of the character of so called Cyanobacteria (Saadi Sulaiman, 2006), they are to walk the path of the bacteria in the fact that the alcoholic extract inhibitor of orange is very effective against bacteria (Ghalibi ,2013) .

Plant also contains a number of phenolic compounds such as Caffic acid(Lu et al., 2002) which has shown to be effective against bacterial and against fungal (Cowan, 1999). The effectiveness of the alcoholic extract was due to the synergistic action of a group of chemical compounds such as phenols, flavonoids and alkaloids present in the extract and a different side chains, giving it the flexibility to work on several targets from microscopic cell (Hugo & Russell, 1987) has pointed Reed (1995) to the ability of these compounds on the deposition of proteins and so by the composition of hydrogen bonds between the hydroxide cyclic groups and proteins, thus inhibiting an enzyme necessary for the metabolism microorganism. Algae differ among themselves in terms of resistance to him for alcoholic extract of orange, as expressed *Microcystis* sp Resistant more than the other algae in response to different concentrations of the alcoholic extract of orange reason may be due to the presence of the genes of the virulent organism poison gives him recipe the direction of the resistance effects.

CONCLUSIONS

It became clear from this study that alcoholic extract of orange a broad impact on microbiology (algae) and different algae with each other in terms of him resistance to extract alcoholic orange, where showed Microcystis sp Resistant more than the other algae response to different concentrations of the extract alcoholic orange. We recommend further studies on the orange and extract effective materials and isolate them to increase their impact on the algae, especially poisonous ones.

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